fadD,fadL PCR Protocol:

a. Using TaKaRa LA Taq™ kit

1. Prepare a mastermix for the number of reactions in 0.5 PCR tube on ice

Reaction mixture for 1 reaction

TaKaRa LA Taq (5 units/µl)	0.2 ul
10X LA PCR Buffer II	2 ul
dNTP Mixture (2.5mM each)	4 ul
Template (<500 ng)	2 ul
Forward Primer (0.2 - 1.0 µM final conc.)	1 ul
Reverse Primer (0.2 - 1.0 µM final conc.)	1 ul
$MgCl_2$ (25mM)	2 ul
Nuclease-Free Water	Up to 20 ul
Total	20 ul

2. Gently flick the PCR tube to mix the reaction mixture and spin down to collect drops.
3. Incubate the tubes in the thermal cyclers using the profile as follows.

PCR profile

Step	**Temperature,°C**	**Time, min**	**Number of cycles**
Initial denaturation	98	1	1
Denaturation	98	0.5	10
Annealing	50	0.5	
Extension	72	2	
Denaturation	98	0.5	20
Annealing	65	0.5	
Extension	72	2	
Final extension	72	6	1
Storage	4	∞	

b. Using TaKaRa Ex Taq™ kit

1. Prepare a mastermix for the number of reactions in 0.5 PCR tube on ice

Reaction mixture for 1 reaction

TaKaRa Ex Taq (5 units/µl)	0.1 ul
10X Ex Taq Buffer	2 ul
dNTP Mixture (2.5mM each)	1.6 ul
Template (<500 ng)	1 ul
Forward Primer (0.2 - 1.0 µM final conc.)	2 ul
Reverse Primer (0.2 - 1.0 µM final conc.)	2 ul
Nuclease-Free Water	Up to 20 ul
Total	20 ul

2. Gently flick the PCR tube to mix the reaction mixture and spin down to collect drops.
3. Incubate the tubes in the thermal cyclers using the profile as follows.

PCR profile

Step	**Temperature,°C**	**Time, min**	**Number of cycles**
Initial denaturation	98	1	1
Denaturation	98	0.5	10
Annealing	50	0.5	
Extension	72	2	
Denaturation	98	0.5	20
Annealing	65	0.5	
Extension	72	2	
Final extension	72	6	1
Storage	4	∞	